with these effects, intracellular Ca2+ accumulation in response to depolarizing voltage steps to 0 mV (control: 60.8 ± 3.0 nM, n=7) was significantly reduced by AID (29.3 ± 1.6 nM, n=7, P<0.001) and AID W-A (18.6 ± 3.4 nM, n=7, P<0.001) peptides; again representing increased inhibitory AID W-A peptide effects (P<0.05 vs AID). Under these conditions, both AID and AID W-A peptides also attenuated G-protein modulation of Ca2+ current (as induced by 1 μM somatostatin).
These data are consistent with inhibitory CaV2.2 peptides acting to reduce synaptic transmission due to a direct effect on presynaptic VDCCs. The increased inhibitory effect of the AID W-A peptide may represent a starting point to develop inhibitory agents in aberrant CaV2.2 signalling pathways, such as in nociception.

2198-Plat
Ca2+ Signaling Amplification by Oligomerization of L-Type Cav1.2 Channels
Rose E. Dixon, Can Yuan, Manuel F. Navedo, Edward P. Cheng, Luis F. Santana.
University of Washington, Seattle, WA, USA.
Ca2+ influx via L-type CaV1.2 channels is essential for multiple physiological processes including gene expression, excitability, and contraction. Amplification of the Ca2+ signals produced by the opening of these channels is a hallmark of many intracellular signaling cascades, including excitation-contraction (EC) coupling in heart. Using optogenetic approaches, we discovered that CaV1.2 channels form clusters of varied sizes in ventricular myocytes and that physical interaction between these channels via their C-tails renders them capable of coordinating their gating thereby amplifying Ca2+ influx. Light-induced fusion of wild type (WT) CaV1.2 channels with channels carrying a gain-of-function mutation that causes arrhythmias and autism in humans with Timothy syndrome (CaV1.2-TS) increased Ca2+ currents, diastolic and systolic Ca2+ levels, contractility, and the frequency of arrhythmogenic Ca2+ fluctuations in ventricular myocytes. Our data indicate that these changes Ca2+ signaling resulted from CaV1.2-TS increasing the activity of adjoining WT CaV1.2 channels via protein-to-protein interactions. Our data support the novel concept that oligomerization of CaV1.2 channels can control the amplification of Ca2+ influx in excitable cells.

Platform: Interfacial Protein-Lipid Interactions II

2199-Plat
Synaptotagmin C2 Domain Membrane Targeting: Kinetic and Mechanistic Diversity Among Isoforms from Different Cell Types
Devin S. Brandt[1,2], Matthew Coffman[1], Joseph J. Falke[2], **Jefferson Knight**[1,2].
[1]University of Colorado Denver, Denver, CO, USA, [2]University of Colorado, Boulder, CO, USA.
Synaptotagmin (Syt) triggers Ca^{2+}-dependent membrane fusion during secretion via its tandem C2 domains, termed C2A and C2B. The seventeen known human isoforms are active in different secretory cell types, including neurons (SytI and others) and pancreatic β cells (SytVII and others). Here, quantitative fluorescence measurements reveal notable differences in the membrane docking affinities, kinetics, and molecular driving forces for C2A and C2B domains from SytI and SytVII, using vesicles comprised of physiological target lipid mixtures. In agreement with previous studies, the Ca^{2+} sensitivity of membrane binding is greater for both domains from SytVII than for their counterparts in SytI. We demonstrate that for C2A, this increased sensitivity is due to a stronger SytVIIC2A membrane interaction, which involves substantial contribution from the hydrophobic effect. Association and dissociation rate constants for both SytVII domains are found to be significantly slower than their counterparts in SytI. For SytVIIC2A, the dissociation rate constant is ~50-fold slower than SytIC2A and is reminiscent of the $cPLA_2C2$ domain that is known to insert deeply into membranes. Addition of sodium sulfate decreases the dissociation rate of SytVIIC2A but not SytIC2A, further indicating that hydrophobic contacts play a major role in SytVIIC2A membrane docking. Thus, SytVIIC2A docks to membranes via both hydrophobic and electrostatic interactions, while the membrane docking interaction of SytIC2A is predominantly electrostatic. The inclusion of phosphatidylinositol-4,5-bisphosphate (PIP_2) in membrane mixtures leads to increased affinity and slower dissociation for both C2B domains, but has minimal effects on C2A domains. Overall, highly homologous domains from these two proteins exhibit distinct mechanisms of membrane binding that may reflect their functions in different cell types.

2200-Plat
How PIP2 Lipids Regulate the Position and Phosphorylation of the Syntaxin N-Terminus
George Khelashvili[1], Aurelio Galli[2], Harel Weinstein[1].
[1]Weill Cornell Medical College, Cornell University, New York, NY, USA, [2]Vanderbilt University School of Medicine, Nashville, TN, USA.
Syntaxin, a member of the family of soluble N-ethylmaleimide-sensitive factor attachment protein receptor proteins can bind and regulate plasma membrane ion channels and neurotransmitter transporters (NSS). Studies of such mechanisms for the dopamine transporter (DAT) have established the N-terminal segment of Syntaxin as the site of direct interactions, and have shown the critical role of highly charged PIP2 lipids in regulating Syntaxin-DAT interactions. We used a computational approach that combines mesoscale continuum modeling of protein-membrane interactions with all-atom molecular dynamics (MD) simulations to compare conformational states of Syntaxin in complex with PIP2-enriched and PIP2-depleted membranes. Our mesoscale approach is based on non-linear Poisson-Boltzmann theory of electrostatics and diffusion-like Cahn-Hilliard dynamics that makes possible the quantitative tracking of lipid-type demixing in the membrane due to the interaction with the protein. The calculations with this method identified strong electrostatic interactions of specific sites of Syntaxin with PIP2 lipids that diffused to their vicinity. MD simulations of the resulting system established that as many as five PIP2 lipid molecules can simultaneously bind Syntaxin. The attending segregation of PIP2 lipids appears to have a dramatic effect on the positioning of the Syntaxin N-terminal segment with respect to the membrane/water interface. These results are discussed in the context of the suggested role of PIP2 lipids in regulating Syntaxin-DAT interactions by modulating phosphorylation of Syntaxin at its N-terminus.

2201-Plat
Structure and Kinetics of PTEN Tumor Suppressor Association with Lipid Membranes
Siddharth Shenoy[1], Prabhanshu Shekhar[1], Frank Heinrich[1,2], Hirsh Nanda[1,2], Joseph Curtis[2], Katrice King[3], Arne Gericke[3], Alonzo Ross[4], Mathias Lösche[1,2].
[1]Carnegie Mellon University, Pittsburgh, PA, USA, [2]NIST Center for Neutron Research, Gaithersburg, MD, USA, [3]Worcester Polytechnic Institute, Worcester, MA, USA, [4]University of Massachusetts Medical School, Worcester, MA, USA.
PTEN is the second most commonly mutated protein in human cancer [1]. The PTEN-PI3K phosphorylation switch regulates cell growth and survival by controlling the levels of $PI(3,4,5)P_3$ in the plasma membrane. PTEN's association with the membrane is critical for bringing its active site in close proximity to the substrate, $PI(3,4,5)P_3$. The crystal structure of a truncated PTEN was determined [2], however, the structure of membrane-bound PTEN remains unknown. In this work, we perform systematic binding studies of PTEN with membranes and report first steps toward a structural characterization of PTEN associated with bilayers.
We compared the binding affinities of wt PTEN, the truncated PTEN [2] and two point mutants, C124S and H93R, to lipid membranes with various anionic lipid (PS, $PI(4,5)P_2$ and $PI(3,4,5)P_3$) compositions using Surface Plasmon Resonance (SPR). PS and $PI(4,5)P_2$ show strong cooperativity in binding to wt PTEN while $PI(4,5)P_2$ and $PI(3,4,5)P_3$ show independent binding to the catalytically inactive C124S mutant. The H93R mutation is spatially separated from the active site as well as the membrane binding motifs, yet results in altered affinities to PS and $PI(4,5)P_2$. The truncated PTEN mutant has an increased affinity to PS-containing membranes over wt PTEN due to an increase in its net positive charge. Neutron reflectivity (NR) experiments were performed to characterize the structure of the PTEN-membrane complex. We observe minimal penetration of the proteins into the lipid headgroup region, indicating that protein association occurs only with the membrane surface. Small, yet significant differences in the NR profiles emphasize the role the point mutations have in altering PTEN's association with the membrane. Molecular dynamics and coarse-grained simulations are currently being performed to interpret structural and orientational details in the NR data.
[1] Nature (2006) 441, 424-430.
[2] Cell (1999) 99, 323-334.

2202-Plat
Exploring Fluorescence Lifetime and Homo-FRET Measurements to Monitor Lysozyme Oligomerization in Anionic Lipid Membranes: Relation to "Amyloid-Like" Fibril Formation
Ana M. Melo[1], Aleksander Fedorov[1], Manuel Prieto[1], Ana Coutinho[1,2].
[1]CQFM and IN, Instituto Superior Técnico, UTL, Lisbon, Portugal, [2]Departamento de Química e Bioquímica, FCUL, Lisbon, Portugal.

Negatively-charged lipid membranes have been suggested to trigger ''amyloid-like'' fibril formation by several non-amyloidogenic proteins, *e.g.* lysozyme [1]. We aimed to elucidate the factors that govern the formation of these ''amyloid-like'' fibrils and to characterize their structural and dynamical properties. Lysozyme was labeled with Alexa 488 (A488-Lz) and its interaction with POPC LUVs containing 20 and 30 mol% of POPS was studied using both steady-state and time-resolved fluorescence techniques. The variation of the mean fluorescence lifetime of A488-Lz as a function of the surface coverage of the liposomes was quantitatively described by a three-state model that assumes that monomeric lysozyme molecules partition into the bilayer surface and reversibly assemble into oligomers with N subunits ($N \geq 6$) (cooperative partition model). The global fit was done using the partition coefficients previously determined for A488-Lz by fluorescence correlation spectroscopy (FCS) [2] and by taking into account electrostatic effects by means of the Gouy-Chapman theory. To better evaluate the oligomerization state of membrane-bound lysozyme, the steady-state fluorescence anisotropy of A488-Lz was also measured for two different fluorophore labeling. The extent of energy migration between A488-Lz (decrease in fluorescence anisotropy) was adequately described only for $N = 6 \pm 1$ when the binomial distribution of fluorescently-labeled monomers among the oligomers was considered. Finally, the lipid-protein supramolecular complexes formed at a low lipid/protein molar ratio [1] were characterized by fluorescence lifetime imaging microscopy (FLIM). The average fluorescence lifetime of A488-Lz had a uniform spatial distribution on these structures, being much shorter than the values measured for free and membrane-bound monomeric A488-Lz, reporting the aggregated state of lysozyme.

[1] Zhao *et al*. **2004** *Biochemistry* 43: 10302
[2] Melo *et al*. **2011** *Biochim. Biophys. Acta* 1808: 2559

2203-Plat

Effects of Lipids on the Conformation and Aggregation of the Repeat Domain of a Functional Amyloid, Pmel17

Zhiping Jiang, Ryan P. McGlinchey, Candace M. Pfefferkorn, Jennifer C. Lee.
National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD, USA.

Pmel17 is a structural protein involved in melanin synthesis and deposition that forms fibrous striations in melanosomes, acidic organelles where pigmentation occurs. It was recently shown that a fragment of Pmel17 named the repeat domain (RPT, residues 315-444) is responsible for fibril formation in vitro under mildly acidic conditions. (McGlinchey RP. et al., PNAS, 2009; Pfefferkorn CM. et al., PNAS, 2010; McGlinchey RP. et al., JBC, 2010) Moreover, at neutral pH these fibrils disassemble, supporting a highly reversible aggregation/disaggregation process that could be a way for melanosomes to recycle amyloid fibrils. Here, we investigate the conformation and aggregation state of the RPT domain in the presence of membrane mimics, since it is localized in a membranous organelle. Specifically, micelles formed from detergents like sodium dodecyl sulfate or from lipids such as lysolipid as well as phospholipid vesicles were examined. Along with circular dichroism spectroscopy, which reports on the formation of secondary structure, we exploited the sole intrinsic Trp fluorophore (W423) located at the C-terminal region as a site-specific probe of interaction. To determine the specificity of this interaction, we also produced and examined single Trp mutants at the N-terminal region. Because the melanosome is an acidic organelle, we also explored the pH dependence of interaction in detail. Finally, we carried out aggregation experiments in the presence of lipid monomers and micelles in determining their effects on fibril formation.

2204-Plat

Recognition Specificity of Proteins and Biomembranes: A Computational View

Roman G. Efremov[1], Pavel E. Volynsky[1], Darya V. Pyrkova[1], Anastasia G. Konshina[1], Nikolay A. Krylov[1], Vladimir M. Pentkovsky[2].
[1]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russian Federation, [2]Moscow Institute of Physics and Technology (State University), Moscow, Russian Federation.

Cell membranes, including their individual components like membrane-bound proteins and particular lipids, attract a growing attention as very perspective pharmacological targets. Rational design of new efficient and selective compounds modulating activity of biomembranes, requires atomic-scale information on their spatial structure and dynamics under different conditions. Because such details resist easy experimental characterization, important insight can be gained via computer simulations.

We present the results of structural/dynamic computational studies of membrane proteins and peptides with diverse fold, mode of membrane binding, and biological activities: antimicrobial and cell-penetrating peptides, cardiotoxins from snake venom, transmembrane domains of receptor tyrosine kinases. The computational approach combines Monte Carlo simulations in implicit membranes, molecular dynamics in full-atom lipid bilayers, and molecular hydrophobicity potential analysis. The predictive power of the method was proven via testing against high-resolution experimental data.

Despite different structure and mechanism of membrane permeation, in all cases the polypeptide-membrane recognition reveals a prominent ''self-adapting'' character. Namely, the membrane active agents employ a wide arsenal of structural/dynamic tools in order to insert into lipid bilayer and to accomplish their function. Importantly, lipid bilayer of biological membranes plays essential role in the recognition/binding events. In particular, the membrane surface reveals highly dynamic lateral heterogeneities (clusters), which differ in their packing and hydrophobic properties from the bulk lipids. Such a mosaic nature of membranes is tuned in a wide range by the chemical nature and relative content of lipids, presence of ions, etc. This makes possible mutual adaptation of the two amphiphatic systems (peptide and membrane). Such a diversity of the factors important for polepeptide-bilayer interactions assures their efficient and robust binding to cell membranes. Understanding of such effects creates a basis for rational design of new physiologically active molecules and/or artificial membranes with predefined properties.

2205-Plat

Cardiolipins Binding Sites on Respiratory Chain Complexes

Clement Arnarez.
Centre for Lifesciences, Groningen, Netherlands.

Mitochondria are the power plants of the cell. Most of the ATP used by a cell is produced by the respiratory chain located in the inner membrane of mitochondria.

It is now well accepted that the protein complexes forming the respiratory chain assemble into larger structures, the so-called supercomplexes[1]. The lack of cardiolipin (CL), a double charged phosphoslipid composing more than 10% of the mitochondrial membrane, impairs the formation of these supercomplexes[2,3] and affects their functionality in the respiratory chain[4].

To investigate the mechanism by which CLs favor the formation of supercomplexes we have simulated complexes III and IV embedded in POPC bilayers containing CLs. The use of the MARTINI coarse grained force field[5] was necessary to reach the system size and time scale necessary to this study. Most notably we found that CLs present preferential interfaces on both complexes III and IV. This led us to the hypothesis that these interfaces might play a role in the relative orientation of the complexes in the supercomplexes. This was clearly shown by the comparison of the supercomplexes formed during self-assembly simulations of a mixture of the two complexes in a lipid bilayer with and without CLs present.

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[2] Zhang M. et al in Journal of Biological Chemistry 277 (2002).
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[5] Monticelli L. et al in Journal of Chemical Theory and Computation 4 (2008).

2206-Plat

Cholesterol Enhances or Reduces Metarhodopsin II Formation Depending on Bilayer Thickness

Olivier Soubias, Walter E. Teague, Kirk G. Hines, Klaus Gawrisch.
NIH, Rockville, MD, USA.

Cholesterol is one of the most efficient modulators of G Protein-Coupled Receptor (GPCR) function. We monitored the effect of cholesterol on rhodopsin function for a set of bilayers with different hydrophobic thickness. Surprisingly, cholesterol shifts the Metarhodopsin-I (MI)/Metarhodopsin-II (MII) equilibrium toward MII for bilayers thinner than the average length of hydrophobic transmembrane helices (2.7 nm), and to MI for thicker bilayers. In previous work conducted on rod outer segment disks and model membranes, increasing cholesterol concentration always shifted the equilibrium towards MI. It was proposed that the cholesterol effect is primarily related to a tighter packing of lipid hydrocarbon chains which generates a less permissive environment for the formation of MII. To gain deeper insights into mechanisms, we followed changes in lipid-rhodopsin interaction by ^{2}H NMR using deuterated lipids. It was reported by us and the Brown laboratory that an increase of bilayer hydrophobic thickness in the absence of cholesterol favors MII with a turnover to MI for bilayers that are very thick. Indeed, the cholesterol-induced shifts towards MII for thinner membranes correlated nicely with the cholesterol-induced increase of bilayer hydrophobic thickness measured by NMR suggesting that the increase in bilayer thickness by cholesterol plays a major role in controlling the energetics of the MI-MII equilibrium. Furthermore, changes in average